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493.09 CITRUS RESEAR BORGETURAL LIGHT CONFERENCE

> OCT. 18, 1960 PASADENA



UNITED STATES DEPARTMENT OF AGRICULTURE Agricultural Research Service Western Utilization Research and Development Division

PROGRAM AND ABSTRACTS OF PAPERS CITRUS RESEARCH CONFERENCE

October 18, 1960

Fruit and Vegetable Chemistry Laboratory
263 South Chester Avenue
Pasadena, California

This program contains abstracts of papers to be presented. Material in these abstracts should not be reproduced without the permission of the author and organization involved. The report was prepared for those attending the conference and is not for publication or general distribution.

FOREWORD

This Citrus Research Conference is being held to bring to members of the citrus and allied industries in Southern California and Arizona the latest results of research on the chemistry, pharmacology, and technology of citrus fruits and their products carried on by laboratories of Utilization Research and Development, Agricultural Research Service, U. S. Department of Agriculture. The following Divisions are participating in this year's conference.

Western Utilization Research and Development Division; Western Regional Research Laboratory (Division headquarters), 800 Buchanan Street, Albany 10, California

Fruit and Vegetable Chemistry Laboratory, 263 South Chester Avenue, Pasadena, California

Southern Utilization Research and Development Division: U. S. Fruit and Vegetable Products Laboratory, Winter Haven, Florida

U. S. Fruit and Vegetable Products Laboratory, Weslaco, Texas

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STUDIES OF THE ALDEHYDES OF CITRUS OILS

William L. Stanley and S. H. Vannier

Western Utilization Research and Development Division Fruit and Vegetable Chemistry Laboratory Pasadena, California

Fifteen aldehydes have been identified in lemon oil. The terpene aldehydes citronellal, neral and geranial and the normal saturated aliphatic aldehydes n-heptanal, n-octanal, n-nonanal, n-decanal and n-undecanal were identified by isolation and conversion to the 2, 4-dinitrophenylhydrazone and methone derivatives. The derivatives were identified by elemental analyses, mixture melting points with derivatives of known compounds and by comparison of infrared spectra with those of the known compounds. The normal saturated aliphatic aldehydes n-hexanal, n-dodecanal, n-tridecanal, n-tetradecanal, n-pentadecanal, n-hexadecanal, and n-heptadecanal were determined by log retention time plots. Straight line plots were obtained with five different stationary phases.

The major aldehydes in lemon oil were determined by their conversion to water-soluble Girard derivatives and regeneration with formaldehyde, followed by gas liquid chromatography. Only the amounts of n-octanal, n-non-anal, n-decanal, n-undecanal, n-dodecanal, citronellal and citral were considered in this survey. The ratio n-nonanal/n-octanal appeared to be related to fruit growing area. The relative amount of citral present in the aldehyde mixture also appeared to be related to growing areas and to optical rotation. The relationship of citral to rotation may provide a means for determining the addition of citral to lemon oils.

Lime oil resembled lemon oil in the relative abundance of the major aldehydes but contained higher amounts of n-decanal and n-dodecanal. Orange oils and grapefruit oils were similar. These oils, in contrast to lemon oil, were quite low in n-nonanal. Navel orange oils contained more citral than Valencia oils. Chief variation in the major components were in the amounts of n-octanal and n-decanal. The detailed chromatograms of the minor aldehydes were much more complicated for orange oil than for lemon oil.

INVESTIGATIONS OF THE HYDROCARBONS OF LEMON OILS

Robert M. Ikeda

Western Utilization Research and Development Division Fruit and Vegetable Chemistry Laboratory Pasadena, California

The following hydrocarbons have been isolated and identified in lemon oils: α-pinene, β-pinene, sabinene, myrcene, d-limonene, γ-terpinene, terpinolene, and p-cymene. Identification of these compounds was based on comparison of gas liquid chromatography (GLC) retention times and infrared spectra. With the exception of myrcene, identification of these compounds was further verified by nuclear magnetic resonance spectra.

Samples of domestic lemon oils accumulated from 1955 to the present time were analyzed for hydrocarbon content by downward elution on chromatostrips followed by gas liquid chromatography. Wide variations were observed for the relative concentrations of β -pinene and d-limonene in these samples. The optical rotations of the oil samples were found to be directly proportional to the d-limonene content and inversely proportional to the β -pinene content. These phenomena explain the reason for the wide natural variation in optical rotation of lemon oils and provide a convenient means for detecting the addition of partially racemized d-limonene to lemon oils.

Lemon oil samples which had been stored in loosely capped bottles for several months were found to contain higher amounts of p-cymene than freshly prepared and well sealed, stored samples. Samples high in p-cymene were low in γ -terpinene. Evidence was obtained which conclusively demonstrated that γ -terpinene is the major precursor of p-cymene formed during deterioration of lemon oils. p-Cymene is responsible for the so-called "cymie" offflavor that develops in oxidized oils.

The major hydrocarbons in grapefruit and orange oils were found to be d-limonene, myrcene, and \(\alpha\)-pinene (d-limonene represented approximately 98 percent of the hydrocarbon mixture in both oils). Traces of \(\alpha\)-thujene, sabinene, \(\alpha\)-phellandrene, \(\gamma\)-terpinene, and p-cymene were also found in orange oils. Identifications were based on comparison of GLC retention times. The relative abundance of the hydrocarbons in lime oils resembled those in lemon oils.

RECENT DEVELOPMENTS IN FOAM-MAT DRYING

Arthur I. Morgan, Jr. and R. P. Graham

Western Utilization Research and Development Division Western Regional Research Laboratory Albany, California

Foam-mat drying is a new method of producing instant food powders by air-drying stable foams. These foams dry rapidly because of capillary transport of water to a free surface from inside the foam mass. Rapid drying results in high product quality. The dry foam structure promotes instant rehydration of the product. The very low levels of product moisture which are possible are helpful for storage stability.

Many liquid foods, including citrus juice concentrates, can be converted to stable foams by incorporating a small proportion of an edible foam stabilizer. In the case of orange juice, an amount of glyceryl monostearate equal to 1 percent of the orange soilds can be used with good results. Another foam stabilizer, soluble soya protein with methocel, is also useful as an alternative. In either case, a good stiff foam can be produced by passing the food and stabilizer, together with 2-1/2 volumes of gas (air or nitrogen), through a high shear mixer.

The foams have been air-dried in thin layers and also extruded pieces lying on a flexible moving surface. For this method, about 0.1 pound of product per hour per square foot of installed area can be expected. Drying times depend on air temperature and piece thickness. The warmest air practicable with this method is about 160°F. At this temperature, with 1/8-inch pieces, drying times are 15 to 20 minutes. This method is satisfactory for pilot plant use and for larger scale if the right equipment could be designed. It is not as fast or as efficient as could be desired. We have therefore begun to develop other techniques.

The "crater" technique is a way of extending stable foams in the direction of drying-air flow in such a way that the average distance to a free surface from within the foam is minimal. The foam is first extruded as a thin layer on a perforated metal sheet. The sheet with its load of foam moves over an air blast which pierces the foam above each perforation. The foam is spread aside but not blown off the sheet. This pierced or cratered formation is retained because of the stiffness of the foam. The drying air is then passed through the perforations until the foam is dry. The cratered layer of dry foam can then be cooled. It is easily scraped off the sheet and packaged. In the case of citrus products, the cooling and packaging must be done in dehumidified air, although the drying air itself need not be dehumidified. With this technique, about 0.5 pound of product per hour can be obtained from each square foot of installed surface. Furthermore, the surfaces are more durable and can be more conveniently arranged in an airstream than the

flexible surfaces used with the thin layers or extruded pieces.

Design of dryers for foam-mat drying is under study. The best results are obtained by staging the drying air. The warmest and driest air should contact the freshest foam. If the foam and air move in the same direction in the first stage, the most even product temperature will be maintained and surface overdrying will be avoided. About 75 percent of the moisture in a foam could be removed by pausing a minute in each of three positions in a concurrent first stage, with air being introduced at 200°F. The remaining moisture should be removed in a countercurrent second stage so that a very dry product is obtained. Such a second stage might require the trays to pause a minute in each of six positions, with air being introduced at 130°F. This stage might profitably be divided into two sections with the first having a warmer air supply than the second.

These concepts are being used in design of a small-scale, fully automatic and continuous dryer at Albany, California. The stages will be arranged separately as vertical stacks. Elsewhere, on a small commercial scale, plans are being made to use 4-foot-square stainless steel sheets in a single vertical stack, the first stage air entering at the bottom. This concept can be further scaled up in several ways. One such idea involves a square helix of trays winding up through the airstream. These concepts are under study at the present time.

RESEARCH PROGRAM AT WINTER HAVEN

M. K. Veldhuis, In Charge

Southern Utilization Research and Development Division U. S. Fruit and Vegetable Products Laboratory Winter Haven, Florida

FOAM-MAT DRYING

Owen W. Bissett and J. H. Tatum

Commission and the Western and Southern Utilization Research and Development Divisions. Engineers at Western Utilization Research and Development Division, Albany, California, designed the Teflon fibre-glass belt dryer and auxiliary equipment, which have been largely financed by the Florida Citrus Commission. The equipment has been installed at the U. S. Fruit and Vegetable Products Laboratory, Winter Haven. The unit has a belt 3 feet wide which passes through a drying chamber 15 feet long. The capacity is about 8 pounds of dried product per hour. During the past few months the equipment has been undergoing "shakedown" runs and adjustments so that it can be operated under controlled conditions. Preliminary experiments only have been made to date and these will be described.

Drying is done on the belt under a blast of hot air at about 165°F. in 12 to 15 minutes. In order to permit rapid drying, concentrated orange juice is whipped into a stable foam which forms a porous structure during drying. A small amount of an edible stabilizer is added to aid in the formation of a stable foam. The dried product strips easily from the belt of its own accord. When reinforced with "locked-in" orange oil the flavor of the dried product is very satisfactory.

ESTIMATION OF LINALOOL AND ALPHA-TERPINEOL

Lyle J. Swift

Linalool and alpha-terpineol were found to be the principal volatile substances of filtered orangepeel juice capable of affecting flavor in orange juice. A gas-liquid chromatographic method for the simultaneous determination of these compounds was devised. A standard curve was constructed in which the injected amounts of these alcohols were plotted against the areas of the appropriate peaks. In the 5-foot by 1/4-inch column, Carbowax 1540 was used as the immobile phase, and 145°C. as the column temperature. Injected samples were weighed in all cases. Oils were injected directly, but with

aqueous mixtures or juices it was necessary to distill a portion from neutralized juice, extract with ether, and reduce the volume by distillation through a fractionating column before injecting. The areas of the peaks so obtained were measured by a planimeter and the amounts of the alcohols read from the curve. Recoveries from juices and aqueous mixtures ranged from 94 to 107 percent.

The methods were applied to a few commercial samples. Linalool ranged from 0.21 mg./liter in aged canned juice to 2.34 mg./liter in fresh juice from a finisher. Alpha-terpineol ranged from 0.62 mg./liter in freshly canned juice to 5.05 mg./liter in aged canned juice.

TIME-TEMPERATURE TOLERANCE OF FROZEN CONCENTRATED CITRUS JUICES

Theo J. Kew

Time-temperature tolerance studies are being conducted on frozen concentrated orange juice. New facilities for storage and testing have permitted the flavor evaluation of a considerable number of packs. Cloud stability data have also been obtained. Information is also being obtained on the effect of various temperatures and times on the length of time the product will remain stable in the consumer's refrigerator. This work has involved regular commercial and experimental packs of 4-, 5-1/3-, and 6-fold concentration and packs canned with and without vacuum or inert gas in the headspace.

Commercial packs of 4-fold (42° Brix) frozen concentrate are being studied for cloud and flavor stability after storage at 20°, 15°, 10°, and 5°F. When stored above zero, damage was sustained by these concentrates. A loss of subsequent storage life at household refrigerator temperature (40°F.) has been shown to result even though the time of such temperature exposures was such that no change was obvious upon immediate inspection of the concentrates. Storage life was measured by a cloud stability test and flavor. As long as there was no loss in cloud, there was no detectable flavor change. The panel often detected flavor damage when residual cloud life at 40°F. was just exhausted.

Experimental 4-fold (42° Brix), 5-1/3-fold (53.5° Brix), and 6-fold (58° Brix) concentrates made from a single lot of fruit have been compared for behavior on storage. Particular attention was paid to the effect of degree of concentration on flavor stability. Both cloud and flavor stability increased with increased concentration. The taste panel was able to detect a flavor difference between portions stored at 40° and 0°F. for three series of 4-, 5-1/3, and 6-fold concentrates respectively coded M, N, and O; Q, R, and S,; and W, X, and Y; after 8, 14, and 12 weeks; after 2, 6, and 6 weeks; and after 2, 3, and 5 weeks. The average results showed a shorter storage life for the

42° Brix concentrates but no difference between the 5-1/3 and 6-fold products. After a year of storage at 0°F, the taste panel showed no preference between 4-fold concentrates and either of the more concentrated products.

Experimental 4-fold concentrates sealed in air, nitrogen, and under vacuum are being subjected to storage and will be examined for cloud and flavor stability.

STABILITY OF CITRUS SALADS

N. B. Rushing and V. J. Senn

Studies have been conducted on the effects of temperature of storage and of the addition of preservatives on the quality of chilled, mixed grape-fruit and orange sections. Cover syrup containing 0.033 percent and 0.066 percent of either sodium benzoate or potassium sorbate, or a 1:1 mixture of these, was dispensed into pint jars and filled with salad in a commercial plant. Storage was at 0°, 30°, 40°, and 50°F. Sampling was twice weekly at 50°, weekly at 40°, and biweekly at 30°F. Samples at 0°F. were used as controls for taste testing by the triangular method. Microbiological and chemical analyses of the cover syrup were also made at the periods indicated. The panel found all experimental samples, with and without preservatives, spoiled after one week at 50° and 5 or 6 weeks at 40°F. Samples without preservative were judged spoiled after 12 weeks at 30°F. Storage life at the lower level of preservative was about 16 weeks at 30°F. Spoilage at the higher preservative level was not as definite at 16 weeks, at which time the supply of samples was exhausted.

A distinct drop in microbiological population was observed over several weeks at 30°F., followed by a definite rise over the balance of the storage period. Sodium benzoate more effectively inhibited microbial growth than did potassium sorbate. Plate counts could not be used as an indication of spoilage. Storage life was not increased markedly by any preservative at the levels tested. The panel was unable to detect the preservatives at the levels used.

CHEMICAL PROPERTIES OF CITRUS FLAVONOIDS

Robert M. Horowitz and Bruno Gentili

Western Utilization Research and Development Division Fruit and Vegetable Chemistry Laboratory Pasadena, California

A study has been made of the flavonoid composition of Marsh seedless grapefruit collected at various stages of growth. In all samples the principal flavonoid compound was naringin. A second flavonoid, previously unreported in grapefruit, was found to be poncirin. Poncirin had been discovered earlier by Hattori in the trifoliate orange (Poncirus trifoliata) and had been shown by him to be the 7-rhamnoglucoside of 5, 7-dihydroxy-4'-methoxyflavanone (naringenin 4'-methyl ether). Since poncirin is extremely bitter (its bitterness is comparable to that of naringin) it seemed probable that the rhamnoglucose portion of it must be in the form of neohesperidose, rather than rutinose. (Last year it was reported here that bitter flavonone glycosides contain neohesperidose, while the nonbitter ones contain rutinose.) This has now been confirmed by chemical degradation, and poncirin is therefore the 4'-methyl ether of naringin. Analysis of the various samples by a specially developed spectral method showed that the ratio of poncirin to naringin was about 1 to 5, regardless of the time of year that the fruit was collected.

Another phenolic glycoside which we have isolated from grapefruit (and have shown to be present in lemons and oranges) is phloroglucinol beta-D-glucoside ("phlorin"). Phlorin had not been previously recognized as a natural constituent of plants. The isolation of phlorin from citrus is of interest since (a) it would seem to constitute a plausible substrate for browning reactions, and (b) it is reported to produce glucosuria in laboratory animals, though it is far less potent than phloridzin in this respect. We have found that the addition of a small quantity of phlorin to lemon juice enhances the rate of darkening on long standing.

Preliminary experiments on the flavonoid composition of Valencia oranges have been carried out. These will be reported briefly.

STUDIES ON THE METABOLIC FATE OF CITRUS FLAVONOIDS AND RELATED COMPOUNDS

A. N. Booth, M. S. Masri, and D. J. Robbins

Western Utilization Research and Development Division Pharmacology Laboratory, Albany, Calif.

The success of our work on the metabolic fate of flavonoids in the animal body was dependent to a large extent on the excellent work accomplished at the Pasadena Laboratory involving the isolation and characterization of the numerous flavonoids which are now known to be present in citrus. We have now clearly established that the citrus flavonoids are absorbed from the gastrointestinal tract after oral ingestion and furthermore that these substances undergo significant changes in the body prior to excretion in the urine in the form of breakdown products. The major metabolite excreted is m-hydroxy-phenylpropionic acid when hesperidin, diosmin, or eriodictyol is ingested by rats and rabbits. In the case of naringin, the major metabolite is p-hydroxy-phenylpropionic acid. It is obvious that these C₆-C₃ compounds arise from the right-hand ring of the flavonoid molecule. Until recently the fate of the left-hand ring was not known. It has now been established that cleavage of the left-hand ring takes place, after which three two-carbon fragments are split off by beta-oxidation, leaving the C₆-C₃ residue intact.

Since these flavonoids are absorbed and are therefore present in the body fluids, it is not surprising that they have been reported to exert beneficial effects in man. Numerous physiological effects of flavonoids have also been reported in experimental animals. There is fairly good agreement that many of these effects can be correlated with the antioxidant properties of the flavonoid molecule. Thus, a protective effect of flavonoids on labile endogenous substances such as ascorbic acid and epinephrine have been demonstrated. A mechanism of action of flavonoids has been discovered by Masri and DeEds.

Considerable interest and concern are presently being expressed regarding the safety of food additives and pesticidal residues in foods. To date there is no evidence of toxic effects from the ingestion of flavonoids. However, glucosuria (the excretion of sugar in the urine) is a toxic effect that can be produced by the injection of phloridzin, a flavonoid glucoside found in the root bark of many fruit trees. When rats received naringin and prunin by injection, no glucosuria was observed; however, the phenolic glucoside phlorin was weakly positive.

The occurrence of catechol in the urine of cattle and humans ingesting ordinary diets has been reported. Until recently the origin of the catechol was unknown. We have now implicated the aromatization of quinic acid by

intestinal micro-organisms to account for the urinary catechol. Quinic acid is a hydroxy-cyclohexane derivative occurring in many plant materials including citrus.

WHAT DO CONSUMER PREFERENCE TESTS OF CANNED JUICE FROM RED GRAPEFRUIT INDICATE?

Bruce J. Lime

Southern Utilization Research and Development Division U. S. Fruit and Vegetable Products Laboratory Weslaco, Texas

The U. S. Fruit and Vegetable Products Laboratory, Weslaco, Texas, Texas A. and M. College, and the Agricultural Marketing Service, U. S. Department of Agriculture, made cooperative studies of consumer preferences for white and red grapefruit juice, both pulp-fortified and unfortified.

One test was conducted in two areas (Dallas and Houston, Texas) where red grapefruit and grapefruit juices were familiar products. The other test was conducted in an area (Des Moines, Iowa) relatively unfamiliar with redmeated grapefruit. In the familiar area there was a preference for canned red grapefruit juice over canned white grapefruit juice. The reason given for this preference was that the red juice was sweeter, although chemically the red juice showed no difference in sweetness and a blind taste panel could not distinguish the difference between them. This would indicate that in these areas where red grapefruit was familiar there was an association of redness and sweetness.

In the area where red grapefruit was not familiar there was a slight preference for juice from white grapefruit. Pulp-fortification had no effect on the preferences shown for the juices.

The results were based on limited observations in three areas using grapefruit juices of acceptable quality but not the highest quality that could be commercially manufactured.

CHARACTERIZATION OF LEMON JUICES AND CONCENTRATES BY THEIR TOTAL AMINO ACID/TITRATABLE ACIDITY RATIOS

Laurence A. Rolle

Western Utilization Research and Development Division Fruit and Vegetable Chemistry Laboratory Pasadena, California

For the past several years, work has been in progress in this and other laboratories on the chemical composition of lemon juice. So far, efforts have been directed towards a study of the nonvolatile constituents in solution in the juice, including the sugars, flavonoids, amino acids, vitamins, and minerals. In this laboratory the amino acid fraction, which was separated from the juice by ion-exchange chromatography, has been thoroughly examined as a basis for the characterization of lemon juice. Two-dimensional paper chromatograms of more than 300 samples of lemon juice indicated a consistent pattern of free amino acids including aspartic, glutamic, and a-aminobutyric acids; serine, alanine, asparagine, proline, and arginine in major quantities; and a number of others in lesser quantities. Individual amino acids, proline for example, did not appear useful for characterization purposes because of wide biological variations (as high as 2- or 3-fold). On the other hand, a measure of the total amino acids did not show such variations; therefore, total amino acid content was determined for 148 samples of commercial single-strength juice, using the ninhydrin color reaction characteristic of amino acids, and measuring the color spectrophotometrically. This method has a distinct advantage in that it can be applied directly to the juice itself without the necessity of prior ion-exchange treatment. The resulting total amino acid values were plotted as a function of total titratable acidity of the samples, and it appears that there is a good correlation between the two. Thus, this method, supplemented by the two-dimensional pattern mentioned above, results in the establishment of an additional, powerful criterion which may be useful in characterizing lemon juices and concentrates. Its value lies in the fact that it utilizes not only the identity of the component amino acids, but also, and more significantly, the total amino acid content of the sample, and the relationship of this content to citric acid concentration.

LIST OF CITRUS PUBLICATIONS AND PATENTS*

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R. M. Horowitz and Bruno Bentili Nature 185(4709): 319, Jan. 30, 1960.

^{*}Reprints are available at the addresses indicated; patents are available only by purchase at 25 ¢ a copy from the U. S. Patent Office, Washington, D. C.

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